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Proc. R. Soc. B 2014 **281**, 20132944, published 29 January 2014

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Cite this article: Givnish TJ, Montgomery RA. 2014 Common-garden studies on adaptive radiation of photosynthetic physiology among Hawaiian lobeliads. *Proc. R. Soc. B* **281**: 20132944.
<http://dx.doi.org/10.1098/rspb.2013.2944>

Received: 9 November 2013

Accepted: 3 January 2014

Subject Areas:

ecology, evolution, physiology

Keywords:

adaptive crossover, plasticity, photosynthetic light response, phylogenetically structured analyses, reaction norms

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Electronic supplementary material is available at <http://dx.doi.org/10.1098/rspb.2013.2944> or via <http://rspb.royalsocietypublishing.org>.

Common-garden studies on adaptive radiation of photosynthetic physiology among Hawaiian lobeliads

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Species in an adaptive radiation often occupy different habitats so that individuals of each species develop under different conditions. Showing that a radiation is adaptive thus requires evidence that taxa have diverged genetically and that each has an ecological advantage in using particular habitats or resources, taking into account both phenotypic plasticity and phylogenetic relationships among species. Here, we use a common-garden experiment to show that representative species of Hawaiian lobeliads have diverged adaptively in their leaf-level photosynthetic light responses. Across species, plants genetically shifted their photosynthetic physiology with native light regime in accord with theoretical predictions and exhibited adaptive crossover in net carbon gain—that is, species native to a given light regime outperformed others only under conditions similar to those they occupy in the field, with the rank order of species based on photosynthesis per unit leaf mass changing with light level. These findings make a powerful case for adaptation of photosynthetic light responses to native light regimes and, combined with our earlier field studies, provide the strongest demonstration to date for the evolution of divergent adaptations for energy capture in any group of closely related plants.

1. Introduction

The Hawaiian lobeliads (six genera, approx. 130 spp.) are one of the most spectacular examples of adaptive radiation in flowering plants, involving divergence in habitat, growth form, leaf morphology, pollination biology and mode of seed dispersal [1–3]. Since the lobeliads colonized the Hawaiian chain around 13 Ma [4], they have invaded the full range of light environments on moist sites, from open montane bogs to densely shaded forest understoreys [5]. The Hawaiian lobeliads thus form a model system for studying the evolution of photosynthetic adaptations to light availability [5–7]. However, species in this and other radiations often occupy different habitats, and therefore develop under different conditions. Showing that a radiation is adaptive thus requires evidence that trait differences among taxa are not simply induced by the habitat in which they develop (i.e. developmental plasticity), but instead are due to genetic divergence. Moreover, one must also demonstrate that such differences give individual species an ecological advantage in using particular habitats or resources [8,9].

Previously, we showed that 11 Hawaiian lobeliad species, representing all major subclades, display a 13-fold range of variation ($2.3\text{--}30.0\text{ mol m}^{-2}\text{ d}^{-1}$) in the photon flux density (PFD) they receive in their native habitats, and that variation in their *in situ* photosynthetic light responses appears to be adaptive [5,6]. Species growing in sunnier habitats have higher rates of maximum net photosynthesis and dark respiration per unit area, require more light to saturate photosynthesis, and have higher leaf mass per unit area (LMA, g m^{-2} ; see the electronic supplementary material, figure S1), resulting in higher net rates of photosynthesis than other species in bright light and lower rates in dim light, in accord with economic theory (i.e. maximization of net energy gain per unit investment) [10–12]. Computer simulations—in which a species's physiological response to PFD in its native habitat is used to model carbon gain across habitats—suggest a widespread pattern of *adaptive crossover* [5], in which

species have higher rates of net photosynthesis in their native light regime than do species from brighter or more dimly lit habitats [6,11–14].

These findings are consistent with the rich literature on adaptations of equilibrium (or static) photosynthetic light responses to contrasting light regimes [5,8,10,11,15–26]. Such adaptations have only rarely been studied in large numbers of closely related taxa (*Euphorbia* and *Scaevola* [27–29]; *Acer* [30–32]; *Psychotria* [33]; *Sonchus* alliance [34]), and, outside the Hawaiian lobeliads, never for plants whose distributions along natural light gradients have been quantified and whose phylogenetic relationships with each other are well characterized.

Our field studies on equilibrium photosynthetic light responses (see electronic supplementary material, figure S1) suggest that the observed physiological and morphological differences among species of Hawaiian lobeliads are adapted to their native PFD regimes, but they also raise two fundamental questions. First, to what extent are the observed differences among species *genetic*, rather than merely reflecting phenotypic plasticity induced by the same developmental programme under different conditions? Also, to what extent are differences among species *adaptive*, in terms of enhancing a species's carbon uptake—and thus, perhaps, whole-organism growth and competitive ability [11,12]—relative to close competitors in the same habitats? Common-garden studies are needed to answer both these questions. Addressing them is an essential—but often omitted—aspect of studying any adaptive radiation. To our knowledge, the only previous study to address both questions is Schluter's [35] seminal study of the differential performance of stickleback morphs in limnetic versus benthic microsites in Paxton Lake. Yet species in many radiations often occupy different habitats and thus develop under different conditions. Showing that a radiation is adaptive thus requires evidence that taxa have diverged genetically in such a way that each has an ecological advantage in using particular habitats or resources [8,9,12,13,35] (cf. [36]). Such demonstrations should take into account both developmental plasticity and phylogenetic relationships among species.

To address these questions, we studied the steady-state photosynthetic light responses and LMA (g m^{-2}) of six species of Hawaiian lobeliads, grown under four different light regimes (6, 12, 35 and 80% full sunlight) in a common garden. This experimental design permits three critical comparisons. First, comparisons of a single trait (e.g. maximum rate of photosynthesis A_{max}) among species within a light level allow us to identify genetic differences among species in that trait under those conditions. We can then conduct a 'soft' test of whether such differences are adaptive by seeing whether they are related to the light regimes each species inhabits in qualitative accord with economic theory, which stipulates that A_{max} , dark respiration rate (R), light compensation point and LMA should all increase with native PFD [11,12]. In keeping with tradition, we ask this question with A_{max} and R specified as rates per unit leaf area; this approach is justified if costs scale per unit leaf area, as might occur if the root costs required to support transpiration are substantial. Second, comparisons of a single trait within a species across light levels allow us to describe its reaction norm, or pattern of developmental plasticity, within that species. A species's reaction norm for a given trait is considered adaptive if it confers a fitness advantage [37]. Reaction norms may not always be adaptive—that is, they might diverge from the pattern of (presumably adaptive) variation seen across species that

dominate different environments; such maladaptive reaction norms may help to determine where a species will or will not be competitively successful. Third, comparisons of a single trait across species and light levels allow us to detect differences among species in their reaction norms for that trait. In addition, we can compare reaction norms for net photosynthesis per unit leaf mass to conduct a 'hard' test of adaptation by determining whether carbon gain per unit leaf investment is the greatest for species grown in light treatments similar to their native light regimes, with the rank of species in carbon gain shifting among light levels. This is a central prediction of economic theory as expressed by Horn [38], Givnish [12] and Givnish *et al.* [5], and departs from the Björkman *et al.* [10] formulation by focusing on the material costs associated with leaf mass, not leaf area. By contrast, hypotheses that invoke a trade-off between growth and survival across species predict no adaptive crossover, with species native to brightly lit habitats expected to have the highest photosynthetic rates (or, more generally, relative rates of whole-plant growth) under all conditions [17,39].

2. Material and methods

Measurements of gas exchange, LMA and PFD for 11 species of Hawaiian lobeliads in their native habitats were provided and subjected to phylogenetically unstructured analysis by Givnish *et al.* [5]. We grew six of these species—*Brighamia insignis*, *Delissea rhytidisperma*, *Clermontia fauriei*, *Clermontia parviflora*, *Cyanea pilosa* subsp. *longipedunculata* and *Cyanea leptostegia*—from seeds in a hoop greenhouse located in Volcano, HI (1190 m), in the heart of the elevational range of almost all the lobeliads studied. Characteristics of typical habitat and growth form among these taxa are given in the electronic supplementary material, table S1. These six species span the entire range of native PFD levels and form a nearly complete set of the taxa from the *Brighamia*–*Delissea*–*Cyanea*–*Clermontia* (BDCC) clade we studied in the field. We excluded just two species, *Cyanea floribunda* and *Cyanea hirtella*, because their native PFD and photosynthetic parameters in the field were nearly identical to those of *Cy. pilosa* and *Cy. leptostegia*, which we included. The BDCC clade includes 87% of all species of Hawaiian lobeliads.

The greenhouse was open to ambient air at both ends and up to 1 m from the ground along the sides. The greenhouse was divided into four light treatments that provided 6, 12, 35 and 80% full sunlight, using a combination of neutral density shade cloth and a special plastic film supplied by the Mitsui Corporation that adjusted the R:FR ratio (i.e. ratio of red to far-red light). We matched R:FR ratios in our four greenhouse treatments to levels measured in the field using a portable spectroradiometer. We set the R:FR ratio at 0.89 for 80% sunlight, 0.72 for 35% sunlight, 0.62 for 12% sunlight and 0.53 for 6% sunlight. Average 24 h temperatures ranged from 16.5°C in the 6% light treatment to 18.2°C in the 80% treatment. The greenhouse was equipped with an automatic misting system to deliver stored rainwater on a regular basis and maintain high humidity levels. Plants were also hand-watered to maintain a non-limiting water supply. Plants received slow-release fertilizer (Osmocote) in the potting media and weekly foliar fertilizer treatments (Gaviota 60), and were rotated weekly within and among tables in the assigned light-treatment zone. Seedlings were started on a vermiculite/perlite media in covered trays. Plants were transferred to individual pots when 5 cm tall. For the remainder of the experiment, they were grown in cinder soil and transplanted regularly as they grew to minimize pot effects. At the time of gas-exchange measurements, plants were growing in 11.5–20 l pots, were 0.5–1.3 m tall, and were roughly 1.5 years

old. We found no evidence of plants becoming pot-bound at any stage of the study.

For each species \times light treatment in the common-garden study, we measured steady-state photosynthesis (A , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) at 10 PFD levels from 0 to $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ using an LI-6400 photosynthesis system (LI-COR, Lincoln, NB) and fitted the data to the three-parameter Michaelis–Menten model,

$$A = A_{\text{max}}\text{PFD}/(\text{PFD} + k) - R, \quad (2.1)$$

where R is the rate of leaf respiration, k is the PFD required to half-saturate photosynthesis at $A_{\text{max}}/2 - R$ and $A_{\text{max}} - R$ is the maximum rate of net photosynthesis [5,10,11]. Data were fitted to the model using a three-dimensional Newton–Raphson approach for leaves from three to seven individuals (mean of 4.5 ± 0.9 replicates per species \times treatment). We did not calculate instantaneous, leaf-level compensation points because they are very strongly correlated with R , given the constancy of quantum yield over C_3 plants, and because they greatly underestimate the actual amount of light required for zero net growth [5,6,12,14].

Inside the photosynthetic cuvette, air temperature and relative humidity were held within narrow limits close to those experienced by most species (approx. 20° – 25°C , 75% relative humidity); temperature effects on photosynthetic parameters should thus be small, given that measurements were made in the middle of the broad, flat optimum for C_3 plants between 20° and 30°C . In addition, light-response measurements were made on a haphazard rotation of species each day. All measurements were made between 8.00 and 13.00 h. CO_2 concentration of the reference stream was set at 380 ppm. Fits of the photosynthetic data to the model were excellent, with $r^2 > 0.95$ in almost all cases. For each sample, we quantified LMA (g m^{-2}). Tabulated values of LMA and photosynthetic parameters for each species, treatment and replicate, as well as metadata, are available in the electronic supplementary material.

To evaluate whether species differed in their photosynthetic parameters as a function of their native PFD regime, as expected from economic theory [5,11], we fitted LMS regressions to the mean parameter and PFD values across species, transforming the data when necessary to ensure linearity, with separate regressions for each light treatment. Significance was assessed using one-tailed t -tests, given that theory predicts that each of the four photosynthetic parameters measured should increase with PFD. We then evaluated the relationship of each photosynthetic parameter to PFD using the Continuous module in BAYESTRAITS [40,41], which employs a GLS approach to produce a phylogenetically structured (PS) test for patterns of correlated evolution between pairs of metric characters in the context of specific relationships among the taxa studied. One-tailed tests of significance were again employed, in this case based on a likelihood ratio test. Tests assumed a standard random-walk model, with branch lengths obtained from the maximum-parsimony tree of Givnish *et al.* [4], with species not included pruned from the tree. Values of κ and δ were set at their default values of 1; we solved for the optimal value of λ and calculated the significance of the regression of traits on native PFD using a t -test. We used a similar PS analysis to test whether the relationships of A_{max} , k , R and LMA to native PFD seen in the field (see electronic supplementary material, figure S1) are also significant.

Reaction norms for each photosynthetic parameter plotted its mean value for each species as a function of light treatment. We employed a mixed-model two-way ANOVA for each trait to determine whether light treatment, species identity or their interaction had a significant effect on the value of that trait. The mixed model included species, light treatment, species \times light treatment interaction and a random effect of individual nested within treatments. The last term reduced the denominator degrees of freedom to account for the fact that we had a single replication of light treatment applied to multiple plants; this

statistical approach should reduce or eliminate problems associated with a single replication of the light treatment. ANOVAs were conducted in JMP [42].

We tested the hypothesis of adaptive crossover by calculating the expected rate of photosynthesis per unit leaf mass that each species realized under each light treatment. We calculated expected instantaneous A_{mass} using Michaelis–Menten parameters and LMA for each individual sampled, assuming that PFD outside the common-garden greenhouse was 1000, 1500 or $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ of photosynthetically active radiation. In this and the following simulations, we multiplied outside PFD values by each greenhouse light level. Thus, values used for the 6% treatment were 60, 90 and $120 \mu\text{mol m}^{-2} \text{ s}^{-1}$. We also calculated expected daily (24 h) net C gain [5] assuming that PFD outside the greenhouse varied sinusoidally over a 12 h day with a maximum value of 1000, 1500 or $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$. These sensitivity analyses used Michaelis–Menten parameters and LMA for each individual sampled to calculate A_{mass} in 10 min increments throughout a 12 h day. These were then summed to estimate daytime C gain. We then used dark respiration measurements and LMA to calculate night-time respiration for a 12 h night and obtain daily net C gain per unit leaf mass [5,6]. We were unable to calculate net C gain on a whole-plant basis [5,43], because we did not measure stem and root respiration and had limited data on biomass allocation among organs. At each light level in all six simulations, we determined which species had the highest energetic return on investment in leaf tissue, and asked whether the identity of that species changed with light availability and was positively associated with PFD levels in its native habitat. Differences in the estimated rates of carbon uptake by the most productive species versus the ‘runners-up’ indicate only a short-term advantage; compound-interest growth and overtopping would greatly increase the realized advantages of the most productive species. We ran mixed-model ANOVAs on the results of the simulations to determine whether, as expected, there were significant effects of species, PFD and species \times PFD interactions.

Finally, we estimated the rate of divergence in each trait at each light level by setting the ancestral values at the crown of the clade involved (13.2 Ma, based on the bottom-up calibration of [4]) equal to the harmonic mean V^* of the maximum and minimum values, and then assuming constant proportional change in such values. This assumption would minimize the estimated rate of evolution, which can be calculated simply as $\ln(V_{\text{max}}/V^*)/13.2$.

3. Results

(a) Field data

Confirming our previous results based on phylogenetically unstructured analyses, we found that field measurements of k , R and LMA increased significantly with native PFD when the data were analysed in a PS manner using CONTINUOUS (see electronic supplementary material, figure S1). A_{max} also increased significantly with the logarithm of native PFD when analysed in a phylogenetically unstructured manner.

(b) Within-treatment comparisons and ‘soft’ test of adaptation

Eight of 16 comparisons of traits with native PFD across species within individual light treatments reveal significant positive correlations based on PS or unstructured analyses (figure 1; see large asterisks for significance of PS analyses, and equations and small asterisks for significance of ordinary regression analyses). Thus, in half of all cases, we find evidence

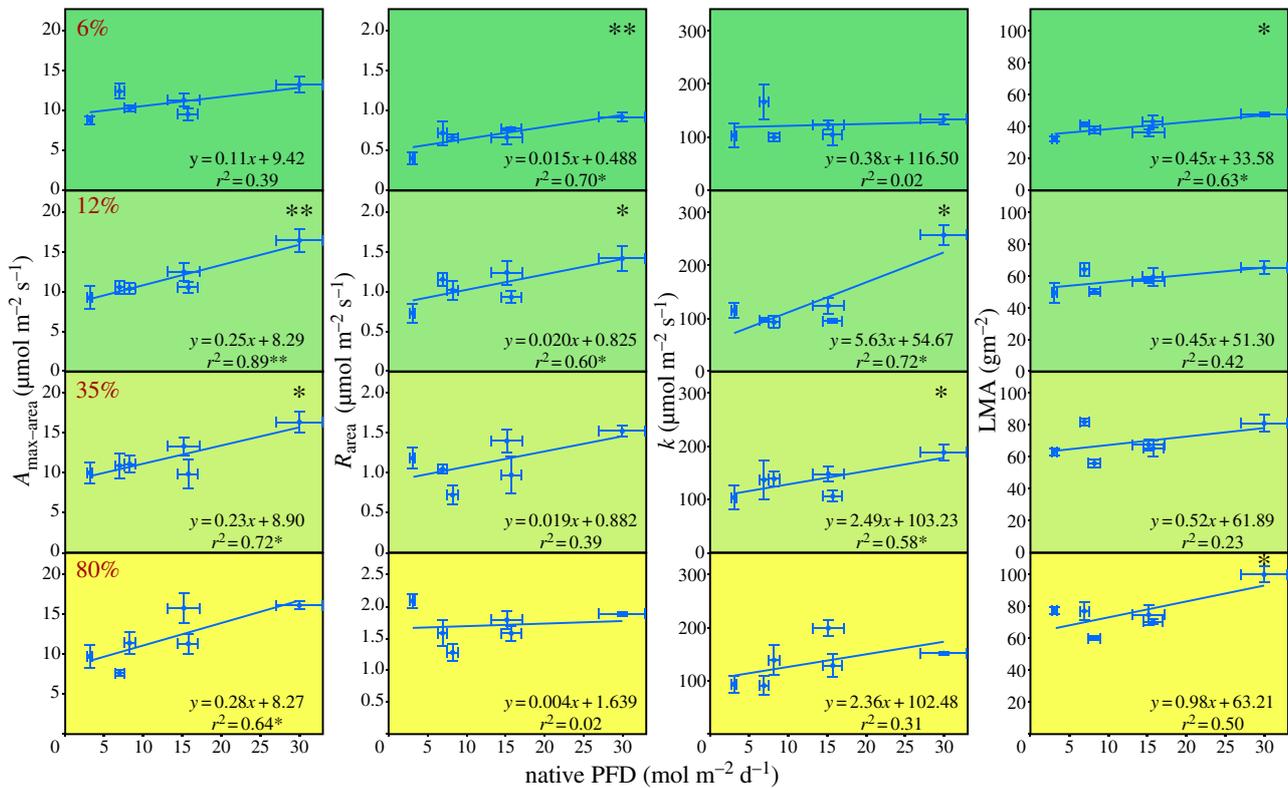


Figure 1. A_{\max} , R , k and LMA in six species of Hawaiian lobeliads grown at 6, 12, 35 and 80% full sunlight in the common garden, plotted against light availability in their native habitat. From left to right, the species are *Cy. pilosa* var. *longipedunculata*, *Cy. leptostegia*, *Cl. parviflora*, *D. rhytidosperma*, *Cl. fauriei* and *B. insignis*. Lines represent LMS regressions of traits on native PFD. Small asterisks adjacent to values of r^2 indicate the significance of traditional regressions; large asterisks indicate the significance of PS analyses of correlated evolution between individual traits and native PFD.

of a genetic component to phenotypic variation among species related to native PFD; the remaining cases also showed the predicted increases in each trait with PFD, but the trends were not significant.

(c) Cross-treatment comparisons and reaction norms

Species identity had a significant effect on all four traits in two-way ANOVAs, while light treatment had a significant effect on R and LMA (figure 2; electronic supplementary material, table S2). There was a significant interaction of species identity and light treatment in effects on k and LMA, while interactions for $A_{\max\text{-area}}$ and R_{area} had p -values of 0.12 and 0.08, respectively. For some traits, reaction norms diverged dramatically from the average trends across species. For example, although $A_{\max\text{-area}}$ increased with native PFD across species in the field (see electronic supplementary material, figure S1) and within common-garden light treatments (figure 1, left column), *Cy. leptostegia* showed a significant decline in $A_{\max\text{-area}}$ as PFD increased across light treatments (figure 2a). All species showed an increase in R_{area} with PFD, with the increase being especially sharp in *Cy. pilosa*, native to densely shaded understoreys (figure 2b). All species show a similar increase in LMA with increasing light availability across treatments (figure 2d).

(d) 'Hard' test of adaptation, adaptive crossover in realized photosynthesis per unit leaf mass

We found significant species and light-treatment effects for instantaneous rates of net photosynthesis per unit leaf mass and daytime C gain (see electronic supplementary material, table S3). P -values for the interaction terms varied from

0.09 to 0.21 for instantaneous rates and from 0.09 to 0.14 for daytime C gain. We found significant species, light treatment and interaction effects for daily net C gain (see electronic supplementary material, table S3). When coupled with the knowledge of native PFD [5] (electronic supplementary material, table S1), the crossing patterns for carbon gain observed in this study (figure 3; electronic supplementary material, tables S1 and S4) provide evidence for adaptive crossover. *Cyanea pilosa*, native to cloud-forest understoreys (mean native PFD = $3.2 \pm 0.4 \text{ mol m}^{-2} \text{ d}^{-1}$), always had the highest rates of instantaneous, daytime and net daily C gain under 6% sunlight (estimated $1.65\text{--}3.3 \text{ mol m}^{-2} \text{ d}^{-1}$ for our three scenarios; figure 3), and significantly higher net daily C gain than *Cy. leptostegia*, *Cl. fauriei* and *B. insignis* in all three scenarios (see electronic supplementary material, table S4). *Clermontia parviflora*, native to gaps in these same cloud forests (mean native PFD = $8.3 \pm 0.8 \text{ mol m}^{-2} \text{ d}^{-1}$), always had the highest rates under 12 and 35% sunlight ($3.3\text{--}19.3 \text{ mol m}^{-2} \text{ d}^{-1}$; figure 3). *Clermontia parviflora* had significantly higher net C gain than *Cy. leptostegia* and *B. insignis* at 12% in all three scenarios, all species at 35% in the $1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ scenario, and all but *D. rhytidosperma* at 35% in the 1500 and $2000 \mu\text{mol m}^{-2} \text{ s}^{-1}$ scenarios (see electronic supplementary material, table S4). *Delissea rhytidosperma*, native to open mesic forest (mean native PFD = $15.3 \pm 2.1 \text{ mol m}^{-2} \text{ d}^{-1}$), had the highest net C gain under 80% sunlight ($22.0\text{--}44.0 \text{ mol m}^{-2} \text{ d}^{-1}$; figure 3), and significantly higher net C gain than *Cy. pilosa* and *Cy. leptostegia* in all scenarios (see electronic supplementary material, table S4). The most productive species had a significantly greater net C gain than all other species pooled at 6% full sunlight ($p < 0.022$), 35% full sunlight ($p < 0.008$) and 80% full sunlight ($p < 0.0008$),

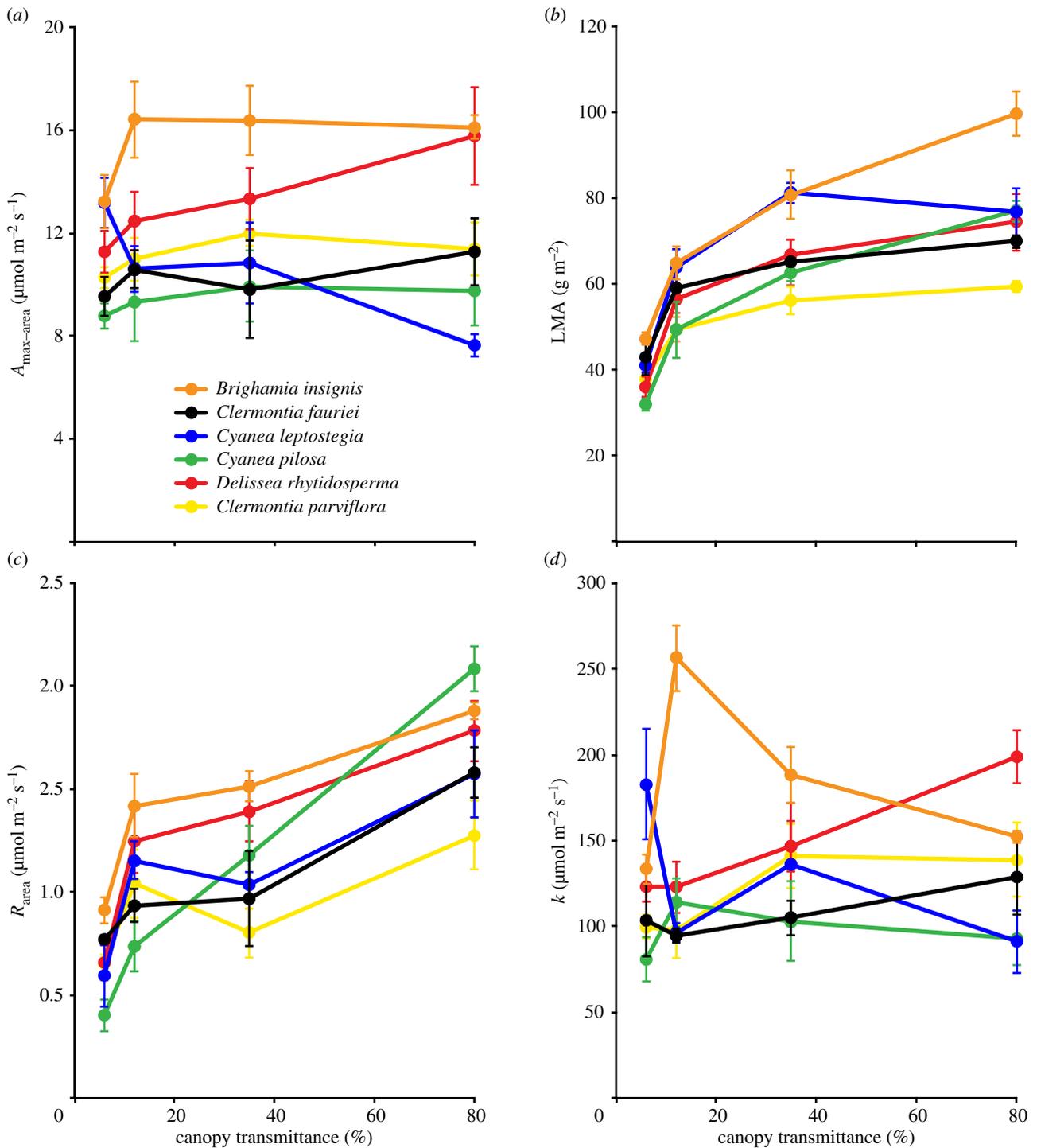


Figure 2. (a–d) Reaction norms for A_{\max} , k , R and LMA of each species as a function of canopy transmittance in the common gardens. The points on each colour-coded curve represent the means for each trait for a given species at 6, 12, 35 and 80% full sunlight; bars represent standard errors about those means.

and a marginally non-significant advantage at 12% full sunlight ($p < 0.052$). Of the six species studied, *Cy. pilosa* was native to the lowest PFD levels ($3.2 \pm 0.4 \text{ mol m}^{-2} \text{ d}^{-1}$), *Cl. parviflora* to the fourth-highest PFD levels ($8.3 \pm 0.8 \text{ mol m}^{-2} \text{ d}^{-1}$) and *D. rhytidosperra* to the third-highest PFD levels ($15.3 \pm 2.1 \text{ mol m}^{-2} \text{ d}^{-1}$), in a virtual tie with second-place *Cl. fauriei* (see electronic supplementary material, table S1).

The mean rate of divergence in each trait, averaged over the four light treatments, was $1.5 \pm 0.4\% \text{ Myr}^{-1}$ for LMA, $2.1 \pm 0.6\% \text{ Myr}^{-1}$ for $A_{\max\text{-area}}$, $3.0 \pm 0.6\% \text{ Myr}^{-1}$ for k and $2.5 \pm 0.5\% \text{ Myr}^{-1}$ for R_{area} (see electronic supplementary material, table S5). This compares with a nearly 10-fold variation in native PFD (see electronic supplementary material, table S1),

implying a mean rate of divergence in PFD of $17.3\% \text{ Myr}^{-1}$ above and below the harmonic mean of $9.8 \text{ mol m}^{-2} \text{ d}^{-1}$ (see electronic supplementary material, table S5).

4. Discussion

Common-garden studies of ecologically diverse taxa remain a critical tool to answer whether observed phenotypic differences among species are *genetic*, rather than merely reflecting a shared pattern of plasticity combined with development under different conditions. Furthermore, in studies of adaptive radiation, common gardens and reciprocal transplants are among the few approaches that can address the extent

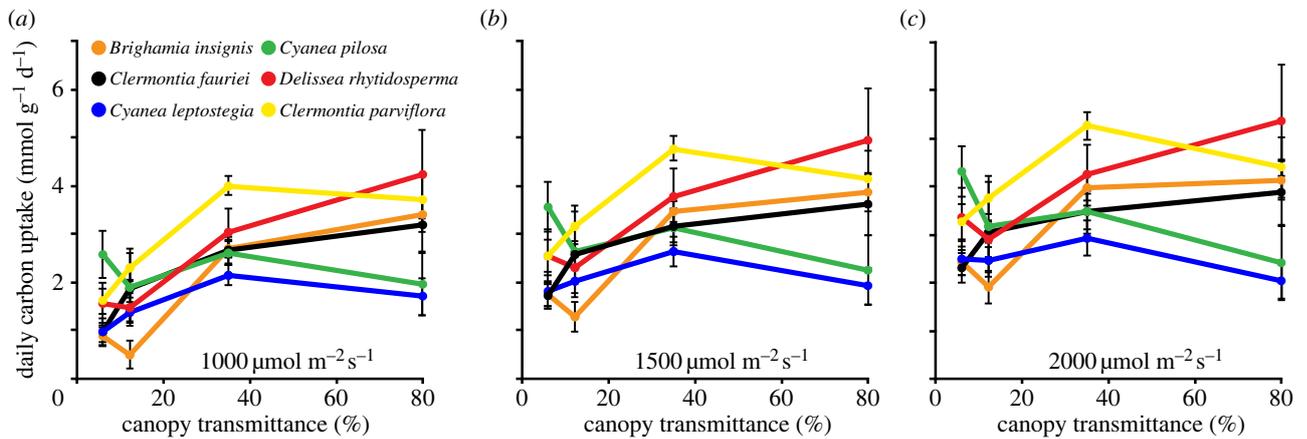


Figure 3. (a–c) Estimated daily net rates of carbon uptake per unit leaf mass for each species as a function of canopy transmittance, assuming peak PFD values of 1000, 1500 or 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The points on each colour-coded curve represent the means \pm s.e. for a given species at 6, 12, 35 and 80% full sunlight. Note the recurrent pattern of adaptive crossover in each simulation, with understory species *Cy. pilosa* outperforming all others at the lowest light level, gap species *Cl. parviflora* outperforming all others at the two intermediate light levels and open-scrub species *D. rhytidosperra* outperforming all others at the highest light level.

to which differences among species are *adaptive*, in terms of enhancing a species's performance relative to close competitors in the same habitats [8,9]. Here, we demonstrate that photosynthetic differences among lobeliad species are at least partly genetic and that they appear to be adaptive. When grown at the same light level, species varied in their photosynthetic physiology in accord with theoretical predictions [11,12]: species from brighter habitats of origin had higher LMA, higher maximum rates of photosynthesis and respiration, and required more light to saturate photosynthesis (figure 1). These results imply a significant genetic component to differences between species in photosynthetic performance in the common gardens, and suggest the same for the differences originally observed among species in different habitats in the field [5] (electronic supplementary material, figure S1). Our findings are unique in evaluating gas exchange in plants grown at several different light levels; the closest approach involved two paradigmatic studies of Hawaiian *Chamaecybe*, which showed that species maintained the same qualitative differences in photosynthetic light responses seen in different habitats in the field when they were grown in a common garden at a single light level [27,29].

The more interesting question is whether differences among species are adaptive, in terms of enhancing a species's carbon uptake—and thus, perhaps, whole-organism growth and competitive ability [11,12]—relative to close competitors in the same habitats. We asked whether species show adaptive crossover in photosynthesis per unit leaf mass after taking into account their differences in reaction norms for various physiological traits. We found rank reversals in performance among species across greenhouse light levels, supporting adaptive crossover (figure 3; electronic supplementary material, tables S3 and S4). The species that naturally occupies the shadiest microsites of the six taxa investigated also had an apparent energetic advantage over the others in the shadiest light treatment; the species that occupies moderately lit, forest-gap microsites had an energetic advantage over others under somewhat brighter light; and the species that occupies relatively open microsites outperformed all others under the brightest light treatment (figure 3). The carbon-uptake advantage of the most productive species over the second most productive species was substantial, averaging $17.5 \pm 6.2\%$ (s.d.) for instantaneous rates (range: 9.8–28.5%)

and $27.2 \pm 12.3\%$ for daily net C gain (range: 13.7–59.8%). Thus, even in cases in which the most productive species did not have a statistically significant edge over the runner-up in daily net C gain, the apparent magnitude of its proportional advantage was substantial and likely to be of immediate competitive significance [5].

The consistency of these results for calculated rates of instantaneous and daily net carbon gain passes the 'hard' test of adaptation. Individual species are distributed along natural gradients of light availability in a way that reflects their relative advantage in photosynthetic rate at different points along an artificial gradient of light availability. Combined with our data from the field [5–7], the findings presented here provide the most compelling evidence to date for adaptation of photosynthetic physiology to light availability in any system. Specifically, our data demonstrate adaptive crossover in photosynthetic rate per unit investment, with closely related species having an advantage when grown in light regimes similar to those they respectively occupy in the field. Demonstration of adaptive crossover in energy capture or fitness is the key to showing that a given radiation is truly adaptive [9], but such evidence is rarely provided [35,44–47] and in some cases the statistical significance of the observed functional crossover remains untested (e.g. [45]). Our findings do *not* support the applicability of models similar to those of Grime [39], which predict no adaptive crossover, to the Hawaiian lobeliads. In broad terms, our study most closely parallels Schluter's [35] classic demonstration that limnetic and benthic morphs of sticklebacks have an advantage in growth rate in limnetic and benthic microsites, respectively. An unusual feature of both studies is that they incorporate developmental plasticity (see also [48]), which we believe is an essential feature of studies of adaptive radiation, given that species in a given radiation often live and develop in different habitats.

We make four observations in closing. First, three of the six species tested appear to be 'losers', in that they failed to outperform others under any light treatment. We doubt that this result reflects our failure to partition the light gradient into a sufficient number of steps, although only additional experiments could demonstrate that. A more likely explanation is that there are other subtle differences among the native habitats occupied by the less productive species that account for their poor performance in well-watered, fertile soils in this

experiment. For example, *Brighamia* grows on sea cliffs, habitats that are more windswept than those occupied by any other species examined, while *Cl. fauriei* grows in boggy forests that are more nutrient-poor than those inhabited by other species. If we were to vary the supplies of moisture and certain critical nutrients as well as PFD in a more extensive common-garden experiment, we would expect that the less productive species would shift ranks and outperform others at points along a multi-dimensional gradient where conditions are more similar to their native habitats.

Second, in many ways our study bridges the common-garden studies of Clausen, Keck and Hiesey [49,50] and traditional approaches in plant physiological ecology. Clausen *et al.* used common gardens to assess: (i) reaction norms and the extent to which they show that the morphological variation within a species across gradients reflects genetic differentiation versus developmental plasticity; (ii) whether such variation is related to the *survival* of reciprocal transplants; and (iii) whether incipient mating barriers exist among such variants. We extend this approach to asking whether the adaptation of species found at different points along a gradient reflects advantages in photosynthesis (or, putatively, growth) relative to other species, and whether different environments exert divergent selection on form and physiology—a crucial prerequisite for adaptive radiation [8,9]. Ideally, such common-garden experiments should be conducted on monocultures and mixtures in natural habitats, but the abundance of recently introduced vertebrate herbivores in Hawaiian forests precluded that approach for the endangered and poorly defended lobeliads.

Third, the proportional rate of divergence in native PFD among the Hawaiian lobeliads studied here—17.3% Myr⁻¹—is remarkable, roughly twice the fastest known rate of morphological change in plants, involving the rise of gigantic flowers in *Rafflesia* from tiny ancestral forms as flower diameter increased by 9.5% Myr⁻¹ (see [51]). The rates at which the maximum and minimum values of LMA,

A_{\max} , R and k diverged from each other—3.0–6.0% Myr⁻¹ (see electronic supplementary material, table S5)—are substantially lower, but these first estimates of the rates of evolution of photosynthetic parameters now call for similar studies involving other traits and lineages.

Finally, photosynthetic rate interacts with a number of other factors—most notably, allocation to leaves versus other organs [43,52,53], respiratory rates of non-photosynthetic tissues [43] and hydraulic adaptations to different rates of evaporative demand in different habitats [53,54]—in determining whole-plant growth, survival and competitive ability across PFD gradients. Our limited data on biomass allocation (R. A. Montgomery 2000–2002, unpublished data) suggest that our interpretation of adaptive crossover based on leaf-level differences is not compromised by strong differences in allocation to leaves versus stems and roots. The only species with a substantially lower allocation to leaves among those studied (the stem succulent *Brighamia*) never achieved better than third place based on leaf performance, and so could not be a top performer under any of the common-garden conditions even if differences in allocation were incorporated. Nevertheless, future studies should integrate biomass allocation, non-photosynthetic tissue respiration and hydraulics with differences in photosynthetic rate to obtain further insights into the ecological distributions and related adaptations of Hawaiian lobeliads. Approaches similar to those we have taken here are an important step toward understanding the context-dependent value of leaf traits and photosynthetic responses *per se*.

Acknowledgements. The authors wish to thank Guillermo Goldstein, Robert Robichaux, Patti Moriyasu and Leilani Durand for their assistance and advice, and David Baum, Robert Robichaux, Lawren Sack, Donald Waller, Colin Osborne and Jennifer Baltzer for comments. The University of Hawaii Agricultural Experiment Station in Volcano kindly provided grounds for our experimental greenhouse.

Funding statement. This research was supported by grants IBN-9904366, IOS-0614813 and DEB-0830036 from the National Science Foundation.

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